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Isolation and Control of Fungal Post Harvest Rot Pathogens of Cucumber (*Cucumis Sativus*) using Ethanolic Leave and Stem Bark Extract of *Jatropha Tanjorensis*

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ABSTRACT:

The study attempted to find out Isolation and Control of Fungi Associated with rot of cucumis sativum using ethanolic leaves and stem bark extracts of Jatropha tanjorensis in Mubi Local Government Area, Adamawa State. Total number of samples of spoilt of cucumber and healthy ones (5) of them were collected from the Mubi market metropolis and transported to botany laboratory Adamawa State University, Mubi. Potatoes Dextrose Agar (PDA) was the culture media used for the isolation of fungi from the sampled cucumber and the preparation of pure culture. A technique was used for the identification of the isolated fungi. Pathogenicity test was carried out in order to know if the isolated fungi were really responsible for the spoilage of the cucumber. The preparation of the ethanolic leave and stem bark extract was carryout by the procedures described. The extract was prepared into three different concentrations ranging from 200-600mg/ml (i.e. 200, 400 and 600mg/ml). The agar plate diffusion method was used to determine the growth of inhibition of fungi isolated by plant extract. The data on the average zone of inhibition produced was analyzed using ANOVA with the help of Statistical Package for Social Sciences (SPSS). Result from the study showed that two (2) pathogens were identified to be responsible for the rot of cucumber in mubi R microsphorus and R-stolonifer. The result further revealed both ethanolic leave and stem bark extract of Jactroph tanjorensis has an effect on both pathogens isolated from the sample but the ethanolic leaves extract is more effective than stem bark it concluded that R-microphorus R-stolonifer are pathogens causing rot in cucumber in Mubi market, Ethanolic leave and stem bark extract was found to be effective in inhibiting the growth of pathogens.

Keywords: Isolation and Control, Fungi, Cucumber, Ethanolic Leave, Stem Bark Extract

1. Introduction

Fruits are the consumable part of mature ovary of flowering plants which are normally eaten raw fruits also includes many structures that are not commonly called fruits such as bean pods, corn kernels, tomatoes and wheat grains (Ikhiwili, 2012). It is impossible to overstate the significance of fruits in human nutrition as they supply vital growth elements like vitamins and minerals required for healthy metabolism (Al-Hindi *et al*., 2011). Fruits are becoming the primary dietary source for both humans and many animals (Lewis, 2022). However, throughout the storage period, fruits often have an active metabolism and are prone to spoiling (Singh *et al.,* 2007). The effective development and survival of different parasitic and saprophytic types of fungus are also facilitated by the low pH, high concentrations of different carbohydrates, minerals, vitamins, and amino acids (Droby 2006). According to yearly studies, 20% of cultivated fruits and vegetables perish. (Barth *et., al* 2009) particularly after harvest (Singh et al., 2007). This has been linked to spoiling fungus, which can be harmful or toxic. (Lewis, 2022), Previous studies have found and isolated toxins-producing fungus from damaged fruits (Al-Hindi et al., 2011). There have been reports of pathogenic fungus in allergy or infection patients (Droby, 2006). Mycotoxins and other poisonous metabolites produced by Aspergillus species can be dangerous to people and animals everywhere (Droby, 2006).

Cucumber (*Cucumis sativus*.) is a major vegetable consumed mainly as salad and pickle. Although *Cucumbers* are used to make pickles all throughout the world, they are mostly eaten as salad in underdeveloped nations like Nigeria. Cucumber's high water content (about 95%) makes it a great cooling vegetable and a strong source of potassium, calcium, vitamin A, and vitamin K. In nature, cucumbers are quite perishable. Since cucumbers are prone to shrivelling, 90–95% humidity should be maintained during marketing, transportation, and storage (Dhall et al., 2010). Postharvest rot disease prevalence, pathogen frequency, and the relative importance of different pathogens as decay-causing agents may differ between geographic regions. This is due to the fact that different environments, crop physiology, harvesting methods, and storage conditions can cause microbial diseases to impact crops in different ways. Therefore, it is crucial to identify the fungal pathogens causing the illness in a given area in order to create an efficient disease management program for Hausa potatoes. Furthermore, the development control techniques are essential for reducing food losses due to microbial assault and preventing microbial spoiling.

Microorganism are found almost everywhere in nature and they are known to destroy fruits in general thereby reducing quality for consumption and profits obtained from their sales. Microorganisms especially fungi cause spoilage of cucumber. Fungi such as *Rhizopus spp. Aspergillus flavus, Mucor spp. Aspergillu fumigatus, Aspergilus niger, Fusarium, Oxysporum* have been reported to be often associated with spoilage of cucumber. Hence the need to drive a means to control fungi growth on the cucumber and promote the shelf life of the cucumber. It is important to know that using chemicals to store cucumber is not advisable as they can be harmful to individuals eating the cucumber. Therefore, the present study was undertaken to isolate and control Fungi pathogens on cucumber using *Jactropha tanjorensiv* (Chaya) Leaves and stem back and also to determine the efficacy of leave and stem bark extract *of Jatropha tanjorensis* on the organism isolated.

2. Methodology

Sample collection.

Cucumber was brought from Mubi market and transported to laboratory section of botany department, Adamawa State University Mubi. The cucumber was washed using a distilled water. The cucumber was then be allowed to rot. Using a sterilized knife, the cucumber was then be cut into two pieces. A sterile swab stick was then be used to pick a little portion of the rotten cucumber and inoculated into a potatoes dextrose agar and incubated in the incubator at 37^{O}C for 5 days, after which macroscopic and microscopic examination was then conducted.

Media Preparation.

Potatoes Dextrose Agar (PDA) was prepared according to manufacturer's instruction. 39gm of commercial PDA was poured into 1 liter of distilled water and dissolved. The dissolved media was boiled to 121^{O}c, when boiling the media will be mixed gently, after which the media will be allowed to cool and then poured into petri dish. The media will be allowed to stay for 24hours to determine the sterility of the media.

Pathogenicity Tests

Healthy cucumbers were inoculated with pure cultures of the isolates after being surface sterilized with 0.01% $HgCl_2$ for one minute and washed in five changes of sterile distilled water. The healthy tubers were penetrated with a 2 mm cork borer to a depth of around 2 mm, and the tissue that was drilled was extracted. The excised tissue was then replaced by a 2 mm diameter disc of the culture that had been sliced and inserted into the opening. Sterile Vaseline was used to seal the wound. With the exception of using sterile agar in place of the isolate, the control was set up similarly. Two tubers were infected and two controls were prepared for every isolate. The infected tubers were placed in desiccators and incubated at 30°C under aseptic conditions, following the protocol established by Chimbekujwo (1994) and Basiri et al. (2011). Isolation of any potentially harmful organism was done on a regular basis and compared with the original isolates. A completely randomised design (CRD) was used to set up the studies, and it was duplicated twice. Analysis of Variance was used for data analysis.

Identification of Fungi Isolates

The physical and cultural characteristics of the fungal isolates, including pigmentation, conidial morphology, and colony development pattern, were used for identification. The technique developed by Oyeleke and Manga (2008) was employed to identify the isolated fungus, which involved the use of cotton blue in lactophenol stain. To identify the fungus, a little piece of aerial mycelia was removed using a mounting needle and placed in a drop of lactophenol. Then, a clean slide was stained with the stain. The mycelium was spread out evenly on the slide using the needle. The next step was to insert a cover slip with precision and gently push it down to eliminate any air bubbles. Afterwards, the slide was placed on the light microscope and inspected via the x10 and x40 objective lenses. The fungal organism was recognised based on its appearance and physical traits, in accordance with Adebayo-Tayo et al., (2012), Onuorah et al., (2015) Klich (2002), Samson and Varga (2007).

Preparation and extraction of Jatropha tanjorensis Leave and stem bark.

Jatropha tanjorensis and stem was collected, on a sunny day in order to ensure effective drying, leaves and steam was collected in open mesh orange bags and kept in the shade to minimize photo-oxidative changes. We shall remove any old, insect- or fungus-infested leaves once we have examined the gathered plant material. Plant materials will be dried in a specially designed drying machine with a forced air draft for approximately a week at room temperature (25 °C) until the leaves become brittle enough to break readily. The dried plant material was ground into a fine powder with a diameter of 0.1 mm using a laboratory grinding mill (Telemecanique/MACALAB model 200 LAB). After that, it was sealed in vials and kept out of light. Forty millilitres of progressively polar solvents (technical grade-merek methanol, hexane, dichloromethane, and acetone) will be introduced to four grammes of finely powdered plant material in a polyester plastic tube for every four grammes. The tubes will be shaken violently for three to five minutes at a high-speed using a Labotec model 20.2 shaking machine. The supernants will be decanted into a marked, weighed glass vial after being centrifuged for an additional five minutes at 3500 rpm. The extracts were then mixed after the procedure was carried out three times on the marc. A steam of room temperature cold air was used to remove the solvent. The plant extracts were redissolved in acetone for further phytochemical and microbiological examination.

Phytochemical test:

Test for Flavonoids

The extract was dissolved in alcohol. A single magnesium piece was heated after being soaked in strong hydrochloric acid drop by drop. The presence of flavonoids was indicated by the pink appearance.

Test for Alkaloids

A little amount of extract was filtered after being dissolved in five millilitres of 1.5% v/v hydrochloric acid. Alkaloids were tested using these filtrates.

Test for Tannins

Water was used to warm and filter the test extract. 1 ml of a 5% ferric chloride solution was added to 5 ml of filtrate, and the mixture was allowed to react. Tannin is present if the colour turns dark green or deep blue.

Test for Saponins

In a graduated cylinder, a 1 ml extract solution was diluted with 20 ml of distilled water and agitated for 15 minutes. The formation of steady foam indicates that saponins are present.

Standardization of inoculum.

The isolates were sub-cultured from the stock water suspension on Potatoes Dextrose Agar. Isolates of all members was incubated at 35^{o}c. Fresh, mature cultures (three to five days old) cultivated on potato dextrose agar slants were used to make the inoculum solution. 5 millilitres of distilled sterile water were poured over the colonies. For species of Aspergillus. After carefully rubbing colonies with a sterile loop to create the inocula, the isolates were shaken vigorously for 15 seconds using a vortex mixer before being moved to sterile tube. For fungi that sporulate slowly, like Fusarium and Scedosporium, the suspension was then moved to a sterile syringe that was connected to sterile filter with an 11um (milipore) pore diameter. After filtering, the suspension was gathered in a sterile tube. By removing most of the hypea, this process created an inocolum that was mostly made up of spores.

Determination of Antimicrobial activity of the Jatropha tanjorensis Leave and Steam Bark Extracts

The well agar plate diffusion method was used to determine the growth of inhibition of fungi (isolated) by the leave and stem bark extract as described by (C.L.S I, 2007), three holes of 6.0cm diameter each were made onto the plate with sterile cork borer and filled with 250mg/ml,500mg/ml and 1000mg/ml of the turmeric extract. Extract of the plant, the inoculated plate was allowed to congeal for 30 minutes to allow pre- diffusion time and then incubate at 28°C for 5-7 days. The plate was examined for zone of inhibition (Cheerbrough, 2002). The diameter of zone of inhibition was measured using meter rule and the value obtain was recorded and expressed to the nearest centimeter.

3. Results

Effect of Anti-fungal of Jatropha tanjorensis

The result shows that the concentration of ethanolic stem bark extract of *Jatropha tanjorensis* has the highest zone inhibition on *Rhizophus stolonifer* than *Rhizophus microsporus* having 9.83 ± 0.033^{a} Diameter zone inhibition.

Table 1: *Effect of Antifungal of Jatropha tanjorensis Stem Bark Extract on isolated pathogen*

	Diameter Zone of Inhibition (mm)	
Concentration (mg/ml)	***R. microsporum***	***R. stolonifera***
200	4.60 ± 0.23^{c}	6.90 ± 0.06^{b}
400	6.38 ± 0.04^{b}	7.20 ± 0.12^{b}
600	9.13 ± 0.27^{a}	9.83 ± 0.33^{a}
Control	0.00 ± 0.00^{d}	0.00 ± 0.00^{c}

[Source: Summarized computational output, (2023)]

Means along the column with the same superscript alphabet are not significantly different at $p\leq0.05$.

The Anti-fungal effect of Jatropha tanjorensis leaf extract

The ethanolic leaf extract of *Jatropha tanjorensis* used on the two fungi isolated from pure culture has higher inhibition effect on *Rhizophus stolonifer* than *Rhizophus microsporus* having 10.43 ± 0.10^{a} diameter zone of inhibition.

Table 2: *The Antifungal Effect of Jatropha tanjorensis Leaf Extract*

	Diameter Zone of Inhibition (mm)	
Concentration (mg/ml)	***R. microsporum***	***R. stolonifera***
200	5.38±0.51[c]	7.83±0.25[c]
400	6.55±0.03[b]	9.38±0.27[b]
600	9.03±0.19[a]	10.43±0.10[a]
Control	0.00±0.00[d]	0.00±0.00[d]

[Source: Summarized computational output, (2023)]

Means along the column with the same superscript alphabet are not significantly different at p≤0.05.

Comparison of Anti-fungal effect of stem Bark and Leaf Extract of Jatropha tanjorensis

The leaf and stem bark extract of *Jatropha tanjorensis*, shows that, both the leaf and stem bark of *Jatropha tanjorensis* has effect on both the two pathogens, isolated from pure culture. But comparing the two extracts, the leaf and the stem bark, the leaf has the highest inhibitory effect on both the two pathogens than the stem bark at all concentration.

Table 3: *Shows the Comparison of Antifungal Effect of the Stem Bark and Leaf Extract of J. tanjorensis*

	Diameter Zone of Inhibition (mm)	
Treatment	***R. microsporum***	***R. stolonifera***
Concentration (mg/ml) – (C)		
200	4.99[c]	7.36[c]
400	6.46[b]	8.29[b]
600	9.08[a]	10.13[a]
Control	0.00[d]	0.00[d]
SE±	0.16	0.13
Plant Part – (PP)		
Stem Bark	5.03[a]	5.98[b]
Leaf	5.24[a]	6.91[a]
SE±	0.12	0.09
Interaction		
C x PP	NS	*

[Source: Summarized computational output, (2023)]

Means along the column with the same superscript alphabet are not significantly different at p≤0.05.

Pathogenicity Test

The pathogenicity test obtained from the result showed that both the two fungal pathogens were pathogenic to cucumber (*C sativum*) with varying level of pathogenicity. In the first day of incubation, rot was not observed in all the organisms until the third day. On the third day, there was a significant difference between control and all the fungal pathogen, starting from day three. *Rhizophus stolonifer* has the highest rot diameter from day three, four and five with 30.20cm, 40.45cm, 70.14cm and *Rhizophus microsporus* having the lowest from 20.50cm, 50.35cm, 65.52cm as shown in the table 4.

Table 4: *The Pathogenicity Test*

Day	***R. stolonifera***	***R. microsporum***	**Control**
3	30.20±0.12[b]	20.50±5.77[b]	0.00
4	40.45±0.09[b]	50.35±5.57[a]	0.00
5	70.14±5.81[a]	65.52±3.03[a]	0.00

[Source: Summarized computational output, (2023)]

Means along the column with the same superscript alphabet are not significantly different at p≤0.05.

Interaction Effect of Concentration and Plant part of Jatropha tanjorensis on Rhizophus stolonifer

The interaction effect of concentration and plant part of *Jatropha tanjorensis* on *Rhizophus stolonifer* shows that the leaf extract of *Jatropha tanjorensis* has the highest effect of *Rhizophus stolonifer* at all level of concentration.

Table 5: *The Interaction Effect of Concentration and Plant Part of J. tanjorensis on R. stolonifera*

	Plant Part	
Concentration (mg/ml)	**Stem bark**	**Leaf**
200	6.90	7.83
400	7.20	9.38
600	9.83	10.43
Control	0.00	0.00
SE±	0.19	

[Source: Summarized computational output, (2023)]

Phytochemical test result of Jatropha tanjorensis Leave Stem Bark Extract

Alkaloids

After dissolving the extract in diluted HCL, it was filtered. Mayer's reagent (potassium mercuric iodide) was applied to the filtrate. The presence of an alkaloid was revealed by the formation of a yellow precipitate.

Tannin

After boiling around 8.5g of the extract in 10ml of water in a test tube and filtering it, a few drops of 0.1% ferric chloride were added, and the brownish-green solution revealed the presence of tannin.

Flavonoid

When diluted ammonia (5 ml) and concentrated sulphuric acid (1 ml) were added to a portion of the extract's aqueous filtrate, the presence of flavonoids was revealed by the yellow colouration.

Saponins

5 drops of olive oil were combined with 3 millilitres of each extract in a test tube, and the mixture was shaken vigorously. The presence of saponin was demonstrated by the emulsification that was seen.

Table 6: *Showing the Phytochemical test result of Jatropha tanjorensis Leave Stem Bark Extract*

Phytochemical	**Leave extract**	**Stem Bark extracts**
Saponins	+	+
Tannins	+	+
Alkaloids	+	+
Flavonoids	+	+

[Source: Summarized computational output, (2023)]

Key: + = present, - = absent

Plate 1a: The Pure Culture of *R. stolonifer*

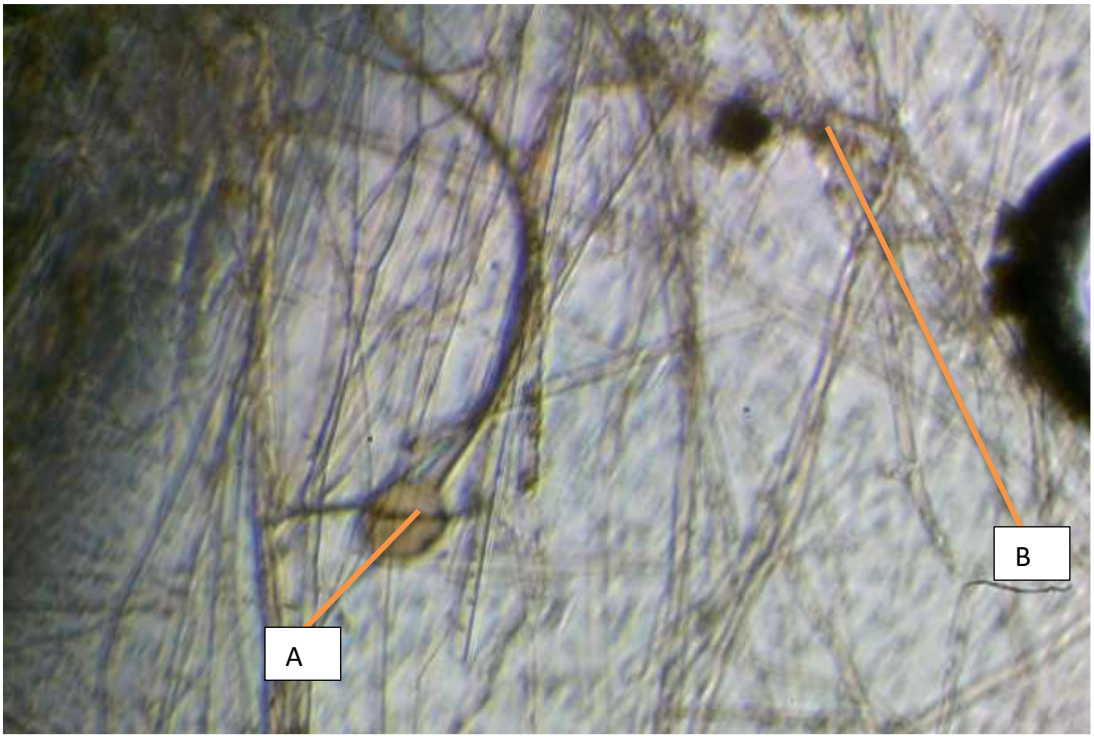

Plate 1b: The photomicrograph of *R. stolonifera* showing (A); hyphae (B); Sporangiospore

Plate 2a: The Pure Culture of *R. microspores*

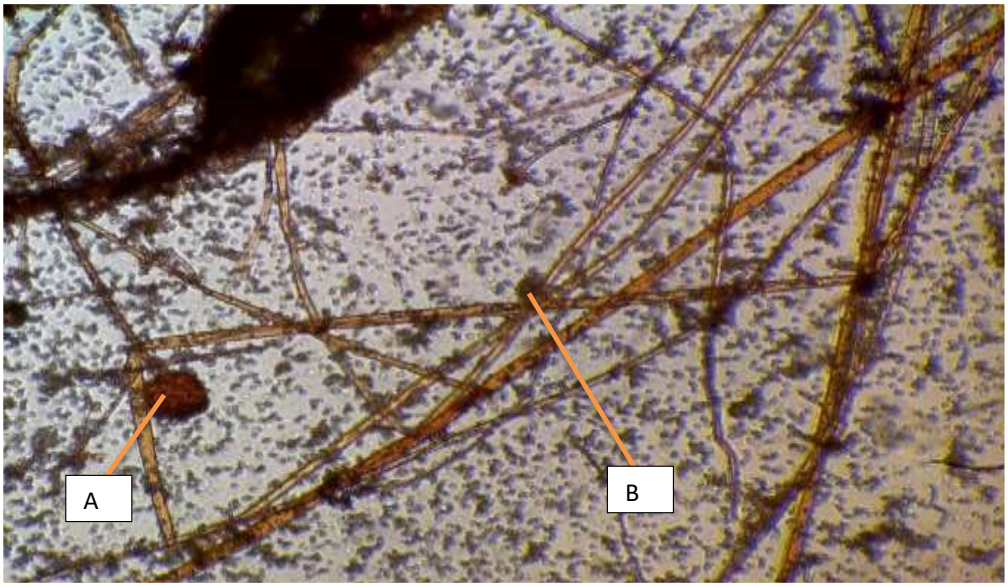

Plate 2b: The photomicrograph of *R. microspores* showing (A); Sporangiospores (B); hyphae

4. Discussion

The qualitative screening of the steam and leave of *Jatropha tanjorensis* showed the presence of most of the compound for which the extract was screened for. Tanis sponins, flavonoids, and trepeniods are some of these substances. Since trepeniods, and flavonoids in particular, have been shown to be active plant components that protect both plants and animals from microbial infection, their presence in the leaf and steam extract of Jatropha tanjorensis validated their antifungal activities. Reicharf (2013). A similar situation was reported by Tizheet et al. (2015) and Ndamito et al. (2013) regarding the solvent extraction method used for the phytochemical constituent extraction, which may explain the inability of the other compound to be present due to its negligible quantity in the extract. In order to support their claim that certain compounds were absent from their plant extracts due to the types of solvents and extraction techniques employed, they conducted quantitative screening on their plant extracts and found the presence of those compounds that the qualitative screening had missed.

The antifungal activity test of the ethanolic leaf and steam extract of *Jatropha tanjorensis* showed significantly higher diameter zone of inhibition in both the test organisms at the highest concentration and the lowest zone of inhibition at the lowest concentration. The existence of those active chemicals found qualitatively in the plant extracts may be the cause of this ethanoic leaf extract's effect-inhibiting properties.

The findings could mean a higher concentration, the higher is the effect of extraction on test organisms. However, the effect of this plant extracts is fungistatic (that is, only stop the growth of the fungi, but does not kill) as proved by the minimize fungicidal concentration (MFC) result of the extract.

The isolation of fungi from Cucumber collected from Mubi Metropolis indicate that there are only two major species (*R. microsporum, R. stolonifera*) that are responsible for the spoilage of Cucumber in Mubi. The finding was authenticated when a fresh and healthy Cucumber were infected with those isolated fungi from the rotten Cucumber, and the infected sample were eventually spoiled. The research found that both leave and steam bark extract of *Jatropha tanjorensis* had significant antifungal activity against *R. microsporum, R. stolonifera*. The result agrees with the finding of Emmanuel (2021) investigated the antifungal activity of leave and steam bark extract of *Jatropha tanjorensis* against fungal species that are common contaminants of Cucumber: *Rhizopus stolonifera*, R. *microsporum, and Aspergillus flavus*.

5. Conclusion

This study was conducted in vitro, so it is not clear whether the antifungal activity of *Jatropha tanjorensis* leave and steam bark extract would be as effective in vivo. However, the results of the study are promising and suggest that *Jatropha tanjorensis* leave and stem bark extract could be a potential natural antifungal agent for the control of *R. stolonifer* and *R. microsporium* and possibly other fungi responsible for the spoilage of cucumber. The pathogenicity test revealed notable growth characteristics of the initial illness sample that led to spoiling. With the isolates exhibiting traits comparable to the original infected samples, the test verified that the rot of cucumbers sold in the Mubi market was caused by the identified fungal pathogen Rhizopus stolonifer and Rhizopus microsporiums. It can be concluded that *Rhizopus microsporium* and *Rhizopus stolonifer* are pathogens causing rot in Cucumber (*Cucumis sativus*) in Mubi Market. *Jatropha tanjorensis* leave and stem bark extract was found to be effective in inhibiting the growth of pathogens. There was increase in inhibition of the pathogen (*R. stolonifera and R. Microsporium*) with the increase in concentration of the *Jatropha tanjorensis leaves and steam* extract. The control leaf of *Jatropha tanjorensis* shows more effect in controlling the pathogens isolated from cucumber (*Cucumis sativum*)

Recommendations

1. Further studies should be conducted using other fungi so as to determine the effect of *Jatropha tanjorensis* leave and stem bark extract on those fungi
2. The use of *Jatropha tanjorensis* leave and stem bark extract should be introduced to farmers to help in the storage of cucumber.

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